REMARKS

Claims 485, 487-502 and 504-507 are pending in the application. Support for "metallic" in claim 485 can be found at *inter alia*, beginning at page 22, line 6 in the Specification. Support for "specific immobilization" can be found at *inter alia*, beginning at page 17, line 26 in the Specification, but is evidenced with implicit support throughout the Specification. Support for the "signaling entity" in claims 505 and 506 can be found at *inter alia*, beginning at page 27, line 1 in the Specification. Support for "specific interaction" in claim 507 can be found at *inter alia*, beginning at page 17, line 26 in the Specification. No new matter has been inserted into the application.

Rejection Under 35 U.S.C. §112, Second Paragraph

Claims 485, 487-502 and 504 have been rejected under 35 U.S.C. §112, second paragraph, as being indefinite. Applicants traverse this rejection. Reconsideration and withdrawal thereof are respectfully requested.

The Examiner has criticized claim 485 for lacking clarity for the phrase "determining the immobilization" of the first and second colloidal particles. The Examiner indicates that an essential step is missing from claim 485 because a step of binding a signaling entity to the "colloid particles/beads" is not recited.

At the outset, the presently claimed invention is directed to determining immobilization of a metal colloid particle to another metal colloid particle (claim 485). Where metal colloids are involved, no special signaling entity is necessary when the assay hinges upon whether one metal colloid is "close in proximity" to the other because the color of the assay solution changes when such metal colloids are "aggregated" rather than homogenously dispersed in solution. A signaling entity would be necessary in the case were non-metal colloid particles are desired to be used because their aggregation do not necessarily change the solution in which they are dispersed (new claim 506). While it is possible to attach a signaling entity to a metallic colloid particle and detect such interaction via the signaling entity, it should not be a requirement that such a signaling entity be linked to the metallic colloid particles. For instance, when gold colloid particles are in close proximity to each other, they emit blue light and as a result the solution is blue colored. When these colloid particles are homogeneously dispersed as happens when there

is no biological or chemical interaction between the chemical or biological species so as to bind to each other, they separately emit pink light and therefore the reaction solution appear pink. Therefore, Applicant submits that a person of skill in the art would be able to detect the immobilization of the metallic colloid particles using whichever technique may be available, with signaling entity or without. Thus, Applicant believes that the language of claim 485 is clear and definite without the need for any additional language related to further binding a signaling entity to the metallic colloid particles.

Rejection Under 35 U.S.C. §103(a) over Mirkin '491 (US 6,984,491) in view of Sigal '670 (US 6,319,670)

Claims 485, 489-502 and 504 have been rejected under 35 U.S.C. §103(a) as being "obvious" over Mirkin '491 in view of Sigal '670. Applicant traverses this rejection. Reconsideration and withdrawal thereof are respectfully requested.

Mirkin '491

Mirkin '491 discloses placing only DNA molecules on colloids. Mirkin '491 discloses only the aggregation of colloids by using the DNA coated colloids. In particular, Mirkin '491 discloses derivatizing gold particles with oligo-compound 1 hybrid molecules, plus compounds 2 and 3, wherein the oligo bears a biotin moiety. The addition of streptavidin, which has four (4) binding sites for biotin, to biotinylated oligos that are attached to gold particles, causes the formation of nanoparticle-streptavidin aggregates. To summarize the Mirkin '491 construct, the gold colloid is bound to the sulfur atoms on the DNA section of the oligo moiety. The biotin is bound to the DNA, and then streptavidin is bound to the biotin.

Mirkin '491 fails to disclose or suggest using self-assembled monolayers (SAM) on the surface of the nanoparticles. Indeed, Mirkin '491 teaches away from using SAM on its nanoparticle surface. Moreover, Mirkin '491 further fails to disclose or suggest determining the immobilization of the first colloid particle with respect to the second colloid particle because the Mirkin '491 construct cannot be used for detecting single interactions between colloid particles between a first chemical or biological species fastened to the first and second colloid particles as in the presently claimed invention.

In particular, with regards to claims 486 and 487, the Examiner refers to Col. 130, lines 9-55 of Mirkin '491 and makes the following statement: "Mirkin teaches that gold particles may be attached to oligonucleotides using biotin labeled oligonucleotides and streptavidin-gold conjugate (affinity tag interaction)."

This is a gross mischaracterization of Mirkin '491 for the following reasons.

First, Mirkin '491 discloses gold particles, which are directly connected to the sulfur atoms on the synthesized oligonucleotides. The Examiner's presumptive "biological or chemical species" in the form of DNA is attached directly to the gold particle. The Examiner's presumptive linker molecules streptavidin-biotin interaction are not linker molecules at all, as these molecules do not contact the gold colloid. The biotin, which is connected to the DNA binds to streptavidin in solution in order to form aggregates of the colloidal particles. Thus, the Examiner has mischaracterized the Mirkin'491 reference. In other words, contrary to Examiner's reading of Mirkin '491, the DNA portion is not linked to the colloid via biotin-streptavidin, but rather biotin-streptavidin is linked to the colloid via the DNA.

The presumptive biological species (DNA) are attached to the gold nanoparticle because it is a DNA-synthetic molecule hybrid with two sulfurs on the synthetic end that chemisorb onto the gold surface. In other words, the DNA are bound directly on to the gold colloid surface because of a sulfur molecule that is present on the synthetic DNA.

In addition, a DNA-biotin molecule is synthesized so that the colloid-DNA-biotin complex will bind streptavidin, which will in turn bind other colloid-DNA-biotin molecules to form a non-specific aggregate. To further clarify, the streptavidin molecule does not contact the gold colloid directly, and in particular, does not act as any chemical or biological agent for effecting any purpose other than as an agent useful for creating colloidal aggregates. Therefore, the DNA molecule, which is the presumptive biological species, in effect serves merely as a linker to the biotin/streptavidin interaction.

The oligonucleotides derivatized with biotin are attached to the gold particle via sulfur atoms on compounds 1, 2 or 3. The streptavidin is added free in solution – not on a nanoparticle. Since each streptavidin has four binding sites for biotin, adding streptavidin free in solution makes nanoparticle-protein aggregates. The DNA-biotin-streptavidin composition of Mirkin '491 builds three dimensional aggregates, which cannot be used in a detection method for

detecting biological interactions. In fact, because streptavidin binds biotin on all four sides, neither the biotin nor the streptavidin is available to participate as an assay ligand.

Second, this type of aggregation method cannot be used to detect a suspected binding interaction because there is only a mass agglomeration engendered by using the biotinylated oligos. Mirkin '491 discloses using this method to form a "gold nanoparticle/protein assembly", and not to detect any suspected interaction among biomolecules.

In contrast, the claimed invention is directed to forming SAMs on nanoparticles that have incorporated into them molecules that bind the surface of the colloid at one end and a binding partner of a biological species at the other end. In a specific embodiment, the presently claimed invention immobilizes a probe chemical or biological species (e. g., DNA or protein) to the gold particle such that a chemical/biological species specific binding between a partner that is attached to another colloid particle is determined. Therefore the inventive molecular construct is distinguished from the Mirkin '491 construct described above.

Sigal '670

Sigal '670 discloses at Col. 7, lines 62-68 that ligands can be adsorbed onto particles that have hydrophobic or charged surfaces such as polystyrene (hydrophobic). Sigal '670 further states that "assay ligands can be adsorbed onto surfaces by modification of the assay ligands with moieties that are known to strongly adsorb on the surface – for example, thiols will facilitate absorption on gold". Sigal '670 appears to require that assay ligands be modified with thiol so as to bind to gold colloid.

Sigal '670 also discloses using a "binding layer". As seen in Col. 8 lines 25-31, Sigal '670 states that a binding layer should have the following characteristics: "should be conductive so should not completely coat the surface and should have defect sites." Example IV of Sigal '670 discloses attaching antibodies via a "binding layer" on gold colloids. The binding layer is disclosed as BSA (bovine serum albumin) that is hydrophobically adsorbed onto the gold. The BSA binding layer is then reacted with NHS-modified signaling molecules and maleimide groups that bind to thiols. Antibodies are separately reacted with Traut's reagents which puts thiol groups on the substrate. The thiol-modified antibody is then reacted with the maleimide modified BSA "binding layer". Example II of Sigal '670 shows adsorption of a probe antibody

onto the colloidal gold by altering the pH of the antibody so that it is non-specifically adsorbed onto the surface through a charge interaction.

Applicant asserts that such a binding layer described in Sigal '670 is not the same as SAM used in the presently claimed method. The present application defines SAM as completely covering the surface.

Mirkin '491 and Sigal '670 are not combinable with each other to arrive at the claimed invention

A person of skill in the art reviewing Mirkin '491 and Sigal '670 references would fail to arrive at the presently claimed invention. First of all, the skilled artisan would modify the Mirkin '491 oligonucleotides with thiol to bind the charged DNA to the charged surface of a gold particle, which Mirkin '491 discloses.

However, the skilled artisan wishing to immobilize proteins to the surface disclosed in Sigal '670, would have been befuddled because it would be impossible to modify the protein with a thiol, or bind hydrophobic proteins to hydrophobic surfaces and charged proteins to surfaces of the opposite charge. In particular, it would have been impossible to synthesize such a protein-thiol hybrid because proteins are too large to be synthesized in such a manner.

Although it may be possible to chemically couple a free thiol to a protein through a primary amine (Lysine) on the protein and a reactive group on the thiol, the large size of the protein would introduce a large entropy component that would make it impossible for the protein to bind to a gold surface via the sulfur group at the end of the thiol. It is known that even thiols comprised of more than 25-30 carbons adsorb onto surface randomly, making the thiol modification irrelevant.

Applicant reiterates. Mirkin '491 makes aggregates. Mirkin '491 discloses no interest in anything else but in making colloidal aggregates. The Mirkin' 491 technical breadth of teaching is narrowly tailored to making such aggregates. A fair reading of the Mirkin '491 teaching cannot lead a person of ordinary skill into creating a colloidal structure that can be used to specifically detect another species. Given this disposition of Mirkin '491, Mirkin '491 fails to disclose or suggest coating its colloid with SAM, which would have allowed for a greater level of detection of specific interaction between biological species. Sigal '670 fails to remedy this difficiency because Sigal '670 fails to disclose or suggest coating any particle with SAM.

Moreover, because Mirkin '491 is narrowly directed to making aggregates, Applicant asserts that applying SAM to the Mirkin '491 colloidal construct does not promote the aggregation of colloidal particles. Rather, naked colloids are significantly more prone to forming aggregates. Therefore, Mirkin '491 discloses a preference for using naked colloids, and teaches away from using SAM or suggests at least the undesirability or the lack of any specific advantage associated with using SAM in forming aggregates. Therefore, even if hypothetically Sigal '670 were to disclose using SAM, this reference still would not be combinable with Mirkin '491 to arrive at the claimed invention.

Therefore, the claimed invention is not obvious over the cited references.

Rejection Under 35 U.S.C. §103(a) over Mirkin '491 (US 6,984,491) in view of Sigal '670 (US 6,319,670) and further in view of Went (US 6,150,179)

Claims 487 and 488 have been rejected under 35 U.S.C. §103(a) as being "obvious" over Mirkin '491 in view of Sigal '670 and further in view of Went '179. Applicant traverses this rejection. Reconsideration and withdrawal thereof are respectfully requested.

Mirkin '491 is discussed above and its deficiencies have been noted above as well.

Sigal '670 is described above and its deficiencies have been noted above as well.

Went '179 is cited for the alleged disclosure of a metal affinity binding tag. However, the Examiner has misread the metal-binding tag-affinity tag interaction. The metal binding tag is a metal complex that binds to an amino acid sequence. One such example is Ni-NTA, which is a metal binding tag, which binds to a (His)6 affinity tag. Applicant notes that the histidine tag affinity tag is a string of amino acids that cannot be attached an oligo (DNA).

For clarity, the metal as part of the affinity tag is not the gold colloid, but rather a type of trace metal and its complex that binds to a metal binding domain of a protein.

In view of the non-combinability of the Mirkin '491 with Sigal '670 references to arrive at the claimed invention as described above, the Went '179 reference fails to provide any remedy for these deficiencies. Therefore, the presently claimed invention is not obvious over the cited references.

Conclusion

It is believed that the application is now in condition for allowance. Applicant requests the Examiner to issue a notice of Allowance in due course. The Examiner is encouraged to contact the undersigned to further the prosecution of the present invention.

The Commissioner is authorized to charge JHK Law's Deposit Account No. 502486 for any fees required under 37 CFR §§1.16 and 1.17 that are not covered, in whole or in part, by a credit card payment enclosed herewith and to credit any overpayment to said Deposit Account No. 502486.

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